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19 March 1999

Documents Management Branch (HFA-305)
Food and Drug Administration
5640 Fishers Lane
Room 1061
Rockville, MD 20852

RE Docket No. 98D-1195

Dear Sir or Madame:

The enclosed comments and suggestions are being submitted regarding the draft guidance entitled: "Bioanalytical Methods Validation for Human Studies", docket #98D-1195. Any requests for further comments or clarification of these comments should be made to

Robert W. Nicholson
Executive Director
PPD Pharmaco Analytical Laboratory
2244 Dabney Road
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Sincerely,

Robert W. Nicholson

Robert W. Nicholson
Executive Director

RWN:jba

Enclosure

98D-1195

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Bioanalytical Methods Validation for Human Studies

Comments and suggestions to the draft guidance (docket No. 98D-1195)
submitted by:
PPD Pharmaco Analytical Laboratory

I. INTRODUCTION

No comments on this section

II. BACKGROUND

Clarification is needed on the statement regarding a need for full validation in the event of major modifications. For example, what is meant by "change of an instrument"? Is this referring to changes in models, components of a modular system, autosampler type etc. In any event, full validation (including all stability testing) is not warranted.

"Linearity" is listed as an essential parameter here, whereas in the Conference Report (1992), "response function" was the analogous term. As the guidance addresses immunological and microbiological methods, and as these methods rarely perform to algorithms that could be considered "linear", "response function" should be maintained whenever a broad definition of all quantitation algorithms is intended.

III. REFERENCE STANDARD

There is actually a fourth common source for reference standards that is not mentioned: the innovator company. This is not analogous to a USP compendial standard, a commercially supplied standard, or a "custom-synthesized" standard.

IV. PRE-STUDY VALIDATION

"Linearity" is mentioned again and this should be changed to "response function".

"Performance parameters" includes "(4) quality control samples". As it is typically precision and accuracy of the quality control samples that are assessed, inclusion of (4) as a break-out point in this paragraph is not necessary.

A. Specificity

The Guidance states that specificity samples should be obtained from six individuals under controlled conditions considering factors "important in the intended study". Since validated assays are generally used for a variety of studies, it is not practical to collect specificity samples that match the intended study with regard to "time of day, food ingestion", etc. It is recommended that the wording

of the guidance be modified so as not to require that the specificity samples be matched to a particular study design. Potential effects from time of day, food ingestion, etc on assay performance should be evaluated by analysis of pre-dose samples collected during the clinical trial.

Reference is made to six individual donors for specificity testing and follows by allowing 10% failure. As it is not possible to reject 10% of six (0.6), it is suggested that the failure rate allow for 1 out of 6.

The guideline suggests that matrix blanks be compared to an aqueous solution of the analyte. It is suggested that matrix blanks should be compared to matrix spiked with analyte at the limit of quantitation.

B. Calibration Curve

Although the scope of the Conference Report (1992) includes immunoassays, it is still common in our industry to prepare calibration curves for immunoassays in a buffer solution. Immunoassays should be held to the same standards as other bioanalytical techniques and it is recommended that language be included in the guidance that clearly states the expectation that immunochemical methods should also employ matrix calibration curves.

There are no criteria suggested for when to increase the number of calibration standards although there are a variety of models in the literature and in practice. This section should not be allowed to imply that there is anything better or worse about using five through eight standards or some other larger number. It should suggest that there be some logical proportionality between the extent of the range and the number of calibration standards used.

Limit of Quantitation (LOQ)

The title of this section should be changed to "Lower Limit of Quantitation (LLOQ), as there are two limits of quantitation per method, a lower and an upper limit

Linearity

This section confuses calibration standard criteria for typical quality control sample criteria. For instance, if the recommendation for a calibration curve is to have from five to eight non-zero standards, how can four of six pass if there are only five to start with? If the ratio of passing calibration standards should be about 66% (or 4/6), it should be stated as a percentage. As the least acceptable number of non-zero standards in a calibration curve is given as five, perhaps the criteria should be "at least 3

of 5 non-zero standards" This could be expressed as 60% of the standards. As this is one of "four factors" that should be met in developing a calibration curve, it should be revisited.

It is stated that four of six non-zero standards should meet the criteria of ≤ 20% at the LOQ and ≤ 15% for other concentrations. Should standards failing this criteria be eliminated from the curve?

C. Precision, Accuracy, and Recovery

The guidance mentions at the end of the precision paragraph that between-day or inter-batch precision "may involve different analysts, equipment, reagents, and laboratories...." As different equipment and reagents have been previously mentioned in the guidance as a possible basis for partial or complete revalidation, this paragraph provides too little information for establishing appropriate experimentation around these variables. Perhaps this language should be omitted altogether or expanded upon in a later section.

If acceptable accuracy and precision are achieved that sufficiently support the human study goals, assays with very low recoveries are acceptable. It is extremely difficult, and meaningless, to determine absolute recovery for methods that include one or several derivatization steps. Although relative recovery can be determined for derivatization-dependent methods, it is not clear why this would be an important assessment of the method when sensitivity, precision and accuracy have been adequately established. It is recommended that requirements to determine recovery be eliminated from the guidance. If recovery measurements are not eliminated from the guidance, there should be no minimum requirements set for acceptable recovery.

D. Quality Control Samples

The requirement for using a different source of biological matrix for each validation batch should be eliminated. Requirements for a different source of matrix for each batch will greatly confound the laboratory's ability to get a meaningful measurement of inter-day variability due to variability related to pool preparations. During analytical studies, new QCs are not prepared for each run so this should not be done during the validation. However, it is recommended that several sources of matrix be pooled to provide the matrix blank used throughout the validation.

What is meant by a "reference standard" in item #7? As this is the first use of the term reference standard in reference to analysis of an authentic standard in a non-biological solution, the reader is left with many questions: (1) are there criteria for choosing a concentration for this reference standard? (2) are there criteria for accepting results obtained from analysis of this reference standard? (3) if the

reference standard is meant to evaluate recovery on a per batch basis, what should happen to batches that pass all other criteria but fail to meet a recovery criterion? (4) is this really a "system suitability" check standard?

The sentence beginning "Percent accuracy is determined by..." should read "Global percent accuracy is determined by...." Percent accuracy, by contrast, should always be defined by dividing individual QC concentrations by their nominal concentrations and multiplying by 100. The more appropriate measure of individual sample accuracy is percent bias. Percent bias is determined by subtracting the nominal concentration from the individual QC result, dividing by the nominal concentration, and multiplying by 100. This provides data that indicates deviation extent (i.e., how far is the result from nominal?) and sign (i.e., does the result deviate higher or lower than nominal?).

E. Stability

Further clarification is needed for the term "container system". This implies that every time a container type changes (polypropylene to polyethylene, plastic to glass, borosilicate glass to soda lime glass, etc.) the stability work needs to be repeated.

Freeze and Thaw Stability – The guidance states that stability QCs should be thawed "unassisted and at room temperature". It should be stated that they are thawed under the same conditions as will be used to thaw study samples. Room temperature thawing is not always the preferred method.

Long-Term Stability – An alternative approach to conducting long term stability should be included. The approach suggested by Dadgar et al to compare stability QCs stored at ≤ -150 °C to a subset of the same pool stored at -20°C is recommended. Since a QC pool is compared against itself at the two storage temperatures, issues related to analytical variation are better controlled and a better evaluation of true stability issues is possible.

Stock Solution Stability – These criteria imply that calibration curves are created on a daily basis from stock solutions. An alternative approach is to prepare frozen pools for each calibration standard in blank matrix for use throughout the validation and the analytical study. This approach is acceptable provided that adequate matrix stability is demonstrated.

F. Acceptance Criteria

This section refers to "between-batch" criteria for precision and accuracy. Criteria should also be stated for "intra-batch" precision and accuracy.

V. IN-STUDY VALIDATION

The sentence "All study samples from a subject should be analyzed in a single run" should be deleted from the guidance. There are many instances when this should not be done due to study design considerations. For instance, when a rising dose tolerance study is being performed, it is usually much more important to provide concentration data quickly on a per dose group basis so that a safe and considered dose escalation can occur.

The terms "run" and "batch" seem to be used interchangeably. These should either be clearly equated in the guidance, should be differentiated, or one term should be chosen and used consistently.

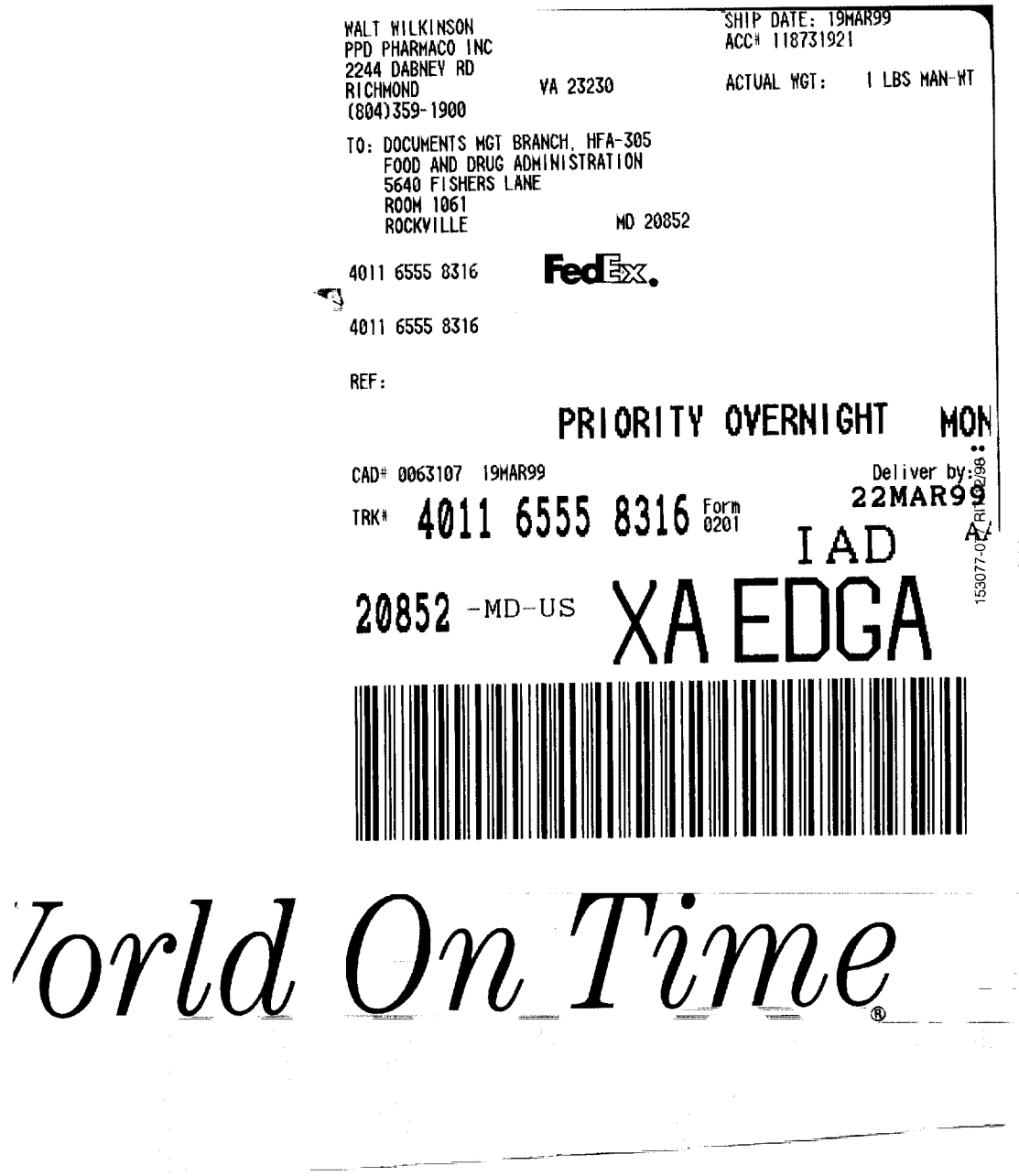
VI. DOCUMENTATION

The requirement for a "bound" laboratory notebook should be eliminated. Bench sheets for recording daily work should be acceptable.

Reassays are often limited by the amount of matrix collected. Frequently, there is not enough sample available to allow for reassay in triplicate. There is also no guidance given on how to interpret or report the results of these multiple assays. This sentence should be eliminated from the guidance or revisited to add further clarification and guidance.

The section on *Documentation for in-study validation* implies that documentation of intra-day accuracy and precision data should be available. This requirement occurs under the bullet for "calibration curves used in analyzing...." This confuses the issue as it is rare that any more than duplicates of each calibration standard are analyzed. Previously, the guidance states that "precision should be measured using a minimum of five determinations per concentration". It is recommended that the requirement for intra-day accuracy and precision as part of the *in-study validation* documentation be eliminated from the guidance.

Clarification is needed on what SOPs and raw data are to be submitted to the Agency. Does the term SOP as used here refer to the analytical method?



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